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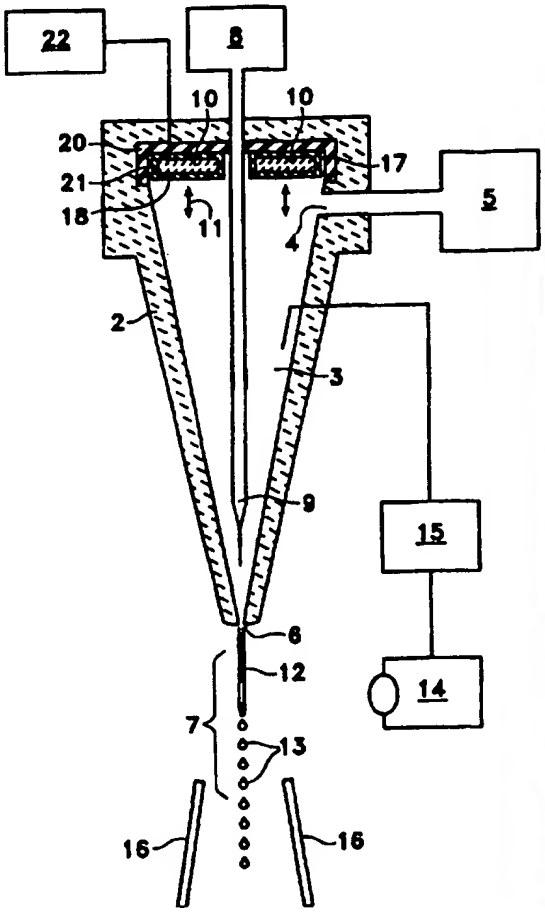
(54) Title: HIGH SPEED FLOW CYTOMETER DROPLET FORMATION SYSTEM

(57) Abstract

A droplet forming flow cytometer system (1) allows high speed processing without the need for high oscillator drive powers through the inclusion of an oscillator or piezoelectric crystal (10) within the nozzle volume (3) and directly coupled to the sheath fluid. The nozzle container (27) continuously converges so as to amplify unidirectional oscillations (11) which are transmitted as pressure waves through the nozzle volume (3) to the nozzle exit so as to form droplets from the fluid jet. The oscillator is directionally isolated so as to avoid moving the entire nozzle container so as to create only pressure waves within the sheath fluid. A variation in substance concentration is achieved through a movable substance introduction port (9) which is positioned within a convergence zone (32) to vary the relative concentration of substance to sheath fluid while still maintaining optimal laminar flow conditions. This variation may be automatically controlled through a sensor and controller configuration. A replaceable tip design is also provided whereby the ceramic nozzle tip is positioned within an edge insert (29) in the nozzle body (24) so as to smoothly transition from nozzle body (24) to nozzle tip (25). The nozzle tip is sealed against its outer surface to the nozzle body so it may be removable for cleaning or replacement.



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HIGH SPEED FLOW CYTOMETER DROPLET FORMATION SYSTEM

I. TECHNICAL FIELD

Generally this invention relates to droplet flow cytometers such as are used for the analysis and sorting of substances contained within separate droplets. Specifically, the invention relates to aspects of such systems which act to form regular droplets after exit from a nozzle orifice.

II. BACKGROUND ART

Droplet flow cytometers have been in clinical and research use for many years. Basically, the systems act to position small amounts of a substance within individual droplets of a sheath fluid. These droplets can be made uniform by utilizing an oscillator which emits a predominant frequency. These oscillations are usually applied to the nozzle container. Since droplet flow cytometry is heavily utilized in both research and clinical environments, such systems have been the subject of much refinement. One of the facets of these systems which has been particularly challenging, however, is the aspect of controlling the drop formation. As to this aspect it has not only been difficult to practically achieve processing rates of much more than 40 kilohertz, it has also been difficult to deal with the incidents of using relatively high power to drive the oscillators involved.

It should be noted that each of the challenges faced in the field of droplet formation for flow cytometers is largely unique to that field. Even seemingly similar fields such as those involving channel-type flow cytometers are not very analogous as they do not face such problems. Their operation as continuous flow devices rather than droplet formation devices makes much of the understandings available in that field inapplicable to the challenges and problems faced in flow cytometry droplet formation systems.

To some degree the challenges for droplet formation may be the result of the fact that although drop formation has been modeled with significant theoretical detail, in practice it still

remains a somewhat empirical subject. While on one level exhaustive mathematical predictions are possible, in practice these predictions can be greatly tempered — and are often revised — by the fact that materials limitations, inherent substance variations, and the like contribute heavily to the end result. A number of "advances" in this field have even proved to be either unnecessary or unworkable in practice.

The level of oscillation energy required in order to achieve uniform droplet formation has, prior to the present invention, been very subject to empirical constraints. This power (often expressed as a voltage amplitude applied to a piezoelectric crystal oscillator) has previously been in the ten volt range. Unfortunately, this relatively high voltage not only results in a need for more robust circuitry, but it also has the undesirable practical consequence of resulting in undesirable electromagnetic emissions. These emissions can impact the sensitivity of the flow cytometer or other nearby equipment. Further, as the desire for higher processing frequencies is pursued, this problem is compounded. Although these problems have been know for years, prior to the present invention it has apparently been an accepted attitude that in order to achieve higher frequencies, still higher oscillation energies are a physical requirement. This invention proves this expectation to be untrue. An example of the extremes to which this rational had been applied is shown in U.S. Patent No. 4361400 to Gray where droplet formation frequencies in the range of 300 to 800 kilohertz had been achieved. This design had required an oscillator powered by approximately 80 volts. The apparent physical requirement of higher powers in order to achieve higher droplet frequencies may have been one reason that most practical droplet flow cytometers operated only in the range of 10 to 50 kHz. The present invention shows that such a relationship is not a physical requirement and, in fact, shows that droplet formation speeds in the 100-200 kHz range are actually possible with only millivolts of power applied to an oscillator.

Yet another problem practically encountered in this field was the challenge of resonances existing within the nozzle assembly. Again, this appears to have simply been accepted as a necessary incident of workable systems and may have resulted in an attitude among those having ordinary skill in the art that it was not practical to vary frequency without unacceptable changes in the performance of the entire system. There also seems to have been some confusion as to the appropriate way to apply the droplet forming oscillations. U.S. Patent No. 4302166 shows that the oscillations are applied to the nozzle container perpendicular to the fluid flow, whereas, U.S. Patent No. 4361400 suggests applying the oscillations to the nozzle container parallel to the lines of flow. In fact, the present invention discloses that each of these systems are suboptimal in that they may even act to generate the resonances and variations in frequency response of the nozzle system.

An even more paradoxical situation exists with respect to the problem of maintaining laminar flow within the nozzle system of a droplet flow cytometer. Although those having ordinary skill in this field have known for years that maintaining laminar flow was desirable, until the present invention, practical systems utilizing replacement tips have not been optimally designed so as to achieve the goal of truly laminar flow. For instance, U.S. Patent No. 4361400 as well as the 1992 publication by Springer Laboratory entitled "Flow Cytometry And Cell Sorting", each show replaceable nozzle tip designs in which laminar flow is disrupted at the junction between the nozzle body and the nozzle tip. Again, such designs seem to present almost a paradox in that they obviously are not optimum from perspective of a goal which has long been known as those having ordinary skill in the art. The present invention not only recognizes this goal but also demonstrates that a solution has been readily available.

Yet another problem encountered in this field is the need to vary parameters to optimize actual conditions encountered in

processing. Again theory and practice did not mix well. While systems were usually designed for optimum conditions, in actual usage such conditions rarely existed. Thus, as U.S. Patent No. 4070617 recognized, designs which allow variation of the substance output velocity within the sheath fluid were desirable. Although such systems permitted some variation, it was recognized that such variations necessarily made conditions within the flow cytometer suboptimal for the simple reason that there is a very definite physical relationship between the sheath substance and drop parameters which must be maintained. Since these parameters are well known to those having ordinary skill in the art (as also indicated in U.S. Patent No. 4302166), the variations required in practice appear to have been accepted as a necessary evil. To some extent, the resulting reduced resolution appears to have been accepted without question. Again, the present invention realizes that approaches which moved conditions away from optimal were not a necessary incident of adapting to conditions practically encountered; it shows that solutions which allow for variation and yet maintain optimal flow conditions are possible.

As explained, most of the foregoing problems had long been recognized by those having ordinary skill in the art. Solutions, however, had either been perceived as unlikely or not been recognized even though the implementing elements had long been available. This may also have been due to the fact that those having ordinary skill in the art may not have fully appreciated the nature of the problem or may have been due to an actual misunderstanding of the physical mechanisms involved. These appear to have included the misunderstanding that actually moving the nozzle was the proper way to induce the droplet forming oscillations and the simple failure to realize that it was possible to coordinate the desire for replaceable nozzle tips with the desire for laminar flow within the flow cytometer nozzle assembly. Similarly, those skilled in the art had long attempted to achieve higher frequency systems which were practically implementable and had attempted to achieve variations which would to the largest extent possible maintain optimal conditions.

Their attempts often led them away from the technical directions taken by the present invention and may even have resulted in the achievements of the present invention being considered an unexpected result of the approach taken.

III. DISCLOSURE OF THE INVENTION

The present invention involves a number of improvements which are applicable to a flow cytometer droplet system. These improvements each offer independent advantages and may be combined synergistically to produce a great increase in the performance of droplet flow cytometers. The preferred embodiment involves a piezoelectric oscillator contained within the sheath fluid above a continuously converging nozzle container. This nozzle container acts to amplify the oscillations which are directly and directionally coupled to the sheath fluid. Further, the location of the substance introduction tube may be adjusted within a convergence zone so as to vary the rate at which the substance is introduced relative to the rate at which the sheath fluid is introduced to maintain optimal conditions. In addition, a replaceable nozzle tip is fit within an edge insert and sealed on its outer surface so as to maintain laminar flow and enhance the amplification of the oscillation throughout the converging nozzle body. As a result of the combination of these various features, the present invention not only achieves practical processing at frequencies of many multiples of typical prior art devices, but it also achieves these processing rates at oscillation powers which are several orders of magnitude less than those typically utilized.

Accordingly, one of the objects of the invention is to provide for a low power system which allows high processing rates. In keeping with this object, one goal is to achieve direct coupling of the oscillations to the sheath fluid and thus minimize any losses associated with material interfaces. In keeping with this object, another goal is to provide for a system which actually amplifies the oscillations so as to produce acceptable fluid variations at the nozzle tip.

Yet another object of the invention is to minimize the impacts of resonance frequencies within the nozzle system. In keeping with this object, a goal is to directionally couple the oscillations to the sheath fluid. It is also a goal to isolate the oscillations from imparting upon the sheath fluid in more than the desired direction.

A further goal of the invention is to provide for a system which allows for the maintenance of laminar flow within the entire nozzle assembly while allowing for both replaceable nozzle tips and for internal variations. The present invention achieves the first object by providing a design which avoids the unnecessary impacts of a seal on the flow condition within the nozzle container. The second object is achieved by providing a system which varies the location at which a substance is introduced while still maintaining optimal, laminar conditions.

Still another object of the invention is to provide for a practically implementable system. In keeping with this object, one goal is to provide a system which can be easily cleaned and for which components can be easily replaced. A goal is also providing a design which can be relatively easily and inexpensively manufactured.

Naturally, further objects of the invention are disclosed throughout other areas of the specification and claims.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic cross sectional view of an embodiment of the invention showing the various features combined.

Figure 2 is a plot of the droplet onset energy of prior art designs compared to that of the present invention.

Figure 3 is a schematic cross sectional view of an alternative design showing the automatic substance adjustment

feature and a directionally coupled, external oscillator.

Figure 4 is a cross sectional view of a replaceable tip design according to one embodiment of the invention.

Figure 5 is a cross sectional view of a prior art replaceable nozzle tip design.

V. BEST MODE FOR CARRYING OUT THE INVENTION

As mentioned, the present invention involves an improved flow cytometer droplet nozzle system which incorporates a variety of features. As shown in figure 1, the flow cytometer system (1) involves nozzle container (2) which establishes nozzle volume (3). Nozzle volume (3) is supplied a liquid by sheath fluid port (4) which acts to introduce a sheath fluid from some sheath reservoir (5). During operation, the sheath fluid flows through nozzle container (2) and out nozzle exit (6) into free fall area (7).

Since the sheath fluid is typically an unreactive substance such as a saline fluid and is an analytically transparent, it has introduced within it some desirable substance such as cells or parts of cells or other items. This substance is maintained in substance reservoir (8) and is introduced to nozzle volume (3) through substance introduction port (9). Through hydrodynamic focusing, the substance flows and is separated into single cell units within the sheath fluid and exits at nozzle exit (6).

In order to form regular droplets, the preferred embodiment utilizes a piezoelectric crystal (10) to cause oscillations (11) within the sheath fluid. These oscillations are transmitted as pressure variations through to nozzle exit (6) and act to allow jet (12) to form regular droplets (13) through the action of surface tension. These processes are well understood and are further explained in a number of references including the 1992 reference entitled "*Flow Cytometry and Cell Sorting*" by A. Radbruch (© Springer-Verlag Berlin Heidelberg) and the 1985 reference entitled "*Flow Cytometry: Instrumentation and Data*

Analysis" edited by Marvin A. Van Dilla, et al. (© Academic Press Inc. (London) Ltd.) each of which are incorporated by reference.

As shown in figure 1, one of the features of the preferred embodiment is the location of piezoelectric crystal (10) within nozzle volume 3. By this feature the oscillator acts to initiate oscillations (11) within the nozzle volume. The oscillator thus may be directly coupled to the sheath fluid. These oscillations are transmitted through the sheath fluid as it flows out nozzle exit (6) and forms droplets (13) below nozzle (6) in freefall area (7). Naturally, although shown to be directly below it is possible that the nozzle assembly could be oriented on its side or in some other relationships and so droplets (13) might form at some other location and yet still be characterized as "below" nozzle tip (6) since they will form in the direction that jet (12) is emitted from nozzle exit (6).

As is well understood, by allowing sheath fluid and the substance to exit from nozzle container (2), cells or cell fragments may be isolated in singular fashion within separate droplets (13) for analysis by sensor (14) which feeds its information to analysis equipment (15). Analysis equipment (15) may provide the necessary data or may act to further process droplets (13) through some equipment such as an electrode in nozzle volume (3) in combination with sorting electrostatic field equipment (16) as is well known in the art. When electrostatic potentials are applied, they may be applied differentially to each droplet based upon the delay in droplet formation. This analysis equipment (15) may also include a separate laser which induces fluorescence and the like in specific cells to allow further sensing and facilitate conducting analysis as well.

As may be easily understood from figure 1, this type of flow cytometer, a droplet flow cytometer, operates quite differently from a channel forming flow cytometer. In channel-type flow cytometers, oscillators and the theories involved are not relevant as no freefall or droplet formation is required.

Further, while the nozzle exit orifice is approximately 50 to 150 microns in diameter in droplet forming flow cytometers, in channel-type flow cytometers, the orifice can be much larger — on the order of 1000 microns. This causes extremely different conditions and has resulted in the two fields being treated somewhat differently by those involved.

Another feature of the invention is how the oscillator couples to actually cause the formation of droplets (13). As shown in figure 1, the oscillator is in this embodiment piezoelectric crystal (10). While, naturally, a variety of different devices could be used in order to achieve oscillation (11), by using piezoelectric crystal (10) a host of different frequencies and powers are possible. It should be understood, however, that while the use of some piezoelectric crystal is usually the preferred technique, the invention should not be considered as limited to that type of oscillator as its teachings can be broadly applied.

As shown in figure 1, piezoelectric crystal (10) is configured as a ring-shaped crystal which occupies most of the top end of nozzle container (2). This ring is mounted directly to nozzle container (2) in a manner so as to be situated within nozzle volume (3). It need not vibrate the nozzle container and, indeed is designed to avoid it. Its oscillations (11) may also be made to occur generally in a direction parallel to the central axis of nozzle container (2) as shown. Further, these oscillations (11) are essentially coupled to the sheath fluid, not to the nozzle container. Thus, rather than taking the directions suggested by some of the prior art involving moving the actual nozzle container, the present invention acts directly upon the sheath fluid to cause pressure variations within the sheath fluid. These pressure variations move down nozzle volume (3) and may actually be amplified by the shape of nozzle container (2) so as to cause surface tensions variations in jet (12) as it emerges from nozzle exit (6). These variations act to pinch off jet (12) and thus form droplets (13). Since the

sheath fluid is not substantially compressible, these pressure variations may pass relatively unattenuated and in fact may be amplified through nozzle volume (3) to achieve the desired droplet formation effect. While others may have considered the desire to coupling directly to the sheath fluid, they failed to recognize ways to do this and did not recognize that they could have positioned the oscillator within the sheath fluid for most efficient coupling.

Both the direct coupling of oscillations (11) to the sheath fluid and the directional nature of the oscillations (11) contribute to the invention's ability to achieve droplet formation at power levels which are several orders of magnitude less than those of the prior art. As may be understood from figure 1, piezoelectric crystal (10) may directly transfer the vast majority of its energy to the sheath fluid. To further enhance the transfer of the majority of the energy into the sheath fluid (rather than the nozzle as often suggested by the prior art), the invention may also incorporate the designing of massive nozzle container elements so as to minimize the transfer of energy through these elements. As may be easily understood, by positioning the oscillator within the sheath fluid, frequency dependencies and resonances which are caused by the vibration of the entire nozzle container can be greatly reduced. Thus, contrary to the teachings of the prior art which suggested vibrating the entire nozzle container, the present invention can specifically avoid such vibrations. This acts to avoid resonance frequencies as might occur through vibrations perpendicular to the lines of flow which may be inevitable whenever the entire nozzle assembly is vibrated. Contrary to those teachings which have suggested mounting the entire nozzle assembly on a flexible membrane so as to allow the entire nozzle assembly to move, the present invention relies not on movement of nozzle container (2) but rather on pressure waves within the sheath fluid in nozzle volume (3). This aspect greatly reduces the amount of power necessary to cause droplet formation and greatly reduces the appearance of resonance frequencies which occur as a result of

the entire vibration of nozzle container (2) among other aspects.

Referring to figure 2, the dramatic impact of these reductions can be understood. Figure 2 shows a conceptual plot of the rough energy of droplet formation onset versus frequency anticipated for the present invention. As shown in figure 2, the energy (expressed in terms of volts applied to a given piezoelectric crystal) is reduced by orders of magnitude. This reduction has been demonstrated for a number of frequencies. As shown in figure 2, the prior art which typically operated in the 10 volt range now only requires ten millivolts or so.

In addition, as shown in figure 2, it can be seen that the prior art was also subject to a great number of resonance frequency variations (shown by the peaks and valleys in the plot of the prior art). These peaks and valleys were to a large extent caused not only by the amount of power required but also by designs which were based upon movement of the entire nozzle assembly rather than merely pressure waves within the nozzle assembly. In sharp contrast to the prior art characteristic conceptually shown in Figure 2, the present invention not only achieves droplet formation with dramatically lower voltages but it also achieves these levels over a relatively large frequency range with very small resonance variations compared to those of the prior art. These relative plots are believed to represent significant differences in result between prior art designs and those of the present invention. While naturally variations will occur due to the particular nozzle designs ultimately chosen, it is believed that through the teachings of the present invention these dramatic variations should be practically achievable in many cases.

Referring again to figure 1, it can be seen that besides merely positioning the oscillator within nozzle volume (3), the embodiment also is designed to minimize the number of material interfaces through which the oscillations must pass before being imparted upon the sheath fluid. While, naturally, it would be

possible to position piezoelectric crystal (10) directly exposed to the sheath fluid, for contamination and other reasons, the preferred embodiment allows for the inclusion of protective coating (17) over piezoelectric crystal (10). This protective coating (17) may actually be some type of epoxy or other coating which has no tendency to interfere either with the sheath material or the oscillations (11) of piezoelectric crystal (10). Again, contrary to the teachings of the prior art which involve numerous material interfaces between the oscillator and the sheath fluid, the present invention minimizes the number of material interfaces through which oscillations (11) must pass. Since any change in material can cause reflection and energy losses, the preferred design allows for only one interface material such as protective coating (17). Thus, only one interface material exists between oscillator surface (18) and the sheath fluid. By positioning piezoelectric crystal (10) within nozzle volume (3) not only can the interface material be limited to the simple epoxy coating mentioned, but also, the oscillator surface (18) can be positioned so as to face directly to the sheath fluid.

As mentioned, another aspect which helps the invention achieve its extraordinary reduction in oscillation drive power is the fact that the oscillator is directionally coupled to the sheath fluid. In order to avoid resonances and energy transmissions in other than the desired direction, the present invention recognizes that unidirectional coupling is desirable. In order to achieve this, as shown in figure 1 the embodiment provides for positioning piezoelectric crystal (10) so that it is detached from the sides of nozzle container (2). Since all piezoelectric crystals act in a manner so as to conserve volume during oscillations, this avoids coupling the inherent perpendicular oscillations to nozzle container (2). Again, through this recognition, the invention can achieve a uniform pressure wave within the sheath fluid. Since oscillator surface (18) is oriented perpendicular to the primary flow direction, the oscillations (11) are coupled substantially only as a flow

direction deemed to be primary, whether the average flow direction, a specific location's flow direction, or even the direction at the nozzle exit (6). This allows for the oscillations to be unidirectionally applied to the sheath fluid and also aids in the reduction of resonance frequencies. As shown in figures 1 and 3, this unidirectional coupling can be achieved through the inclusion of a directional isolator (19). As shown in figure 3, directional isolator (19) may be a separate element such as a rubber or other material which does not transmit frequencies of the predominant oscillation frequency. As shown in figure 1, the directional isolator (19) may actually be spacer (20). Spacer (20) may be a separate element or, as shown in figure 1, may be an integral portion of the top or cap of nozzle container (2) so as to simply act to space oscillator side (21) away from nozzle container (2). The unidirectional coupling of oscillations (11) to the sheath fluid may be enhanced by making oscillator surface (13) planar as shown in figure 1 and by making it cover most of the top surface area. The oscillator is thus established substantially throughout a perpendicular cross sectional area (perpendicular to the primary flow direction) and will cause oscillations throughout it. Thus the ring shaped crystal design coordinates the desire to maximize the surface area of oscillator surface (18) with the unidirectional desire by making it match the typically circular cross section of nozzle container (2). Naturally other shapes can also be used. Further, the coupling, shown in figure 1 and in figure 3 as the portion of the top section of the nozzle container (2) may also be planar and may also be coupled along only one plane. These each contribute to making the main oscillation area cause only one direction of oscillation as can be easily understood.

To further enhance the reduction in oscillation power achievable through the present invention, nozzle container 2 is also designed as a continuously converging nozzle container. This acts to not only maintain laminar flow throughout nozzle volume (3), but also to effectively amplify oscillations (11) as they travel in pressure waves through the sheath fluids from

piezoelectric crystal (10) to nozzle exit (6). As may be understood from figure 1, by continuously converging it is not meant that nozzle volume (3) must constantly or uniformly converge throughout its length, rather, it need only converge at all locations. Thus, nozzle volume (3) has a largest cross-sectional area located at or near its top and has continuously diminishing cross-sectional areas along its length through to nozzle exit (6).

Having a continuously converging nozzle container also helps in maintaining laminar flow up to nozzle exit (6). In this regard nozzle exit (6) should be understood to exist not only at the actual end location of the orifice but more accurately at the point at which there is a significant increase in the pressure gradient so as to make changes in the angle of convergence less important. Unlike the teachings of the prior art which frequently involve straight cylindrical sections within nozzle container (2), this aspect of the invention specifically avoids such possibilities. This is somewhat surprising and may be treated with skepticism by those of ordinary skill in the art because traditional theories provide that once laminar flow is established such flow should continue in most applications when the nozzle container does not expand sharply. In contrast, this aspect of the invention suggests otherwise. While these traditional laminar flow theories may be appropriate in some instances, the continuous convergence of the sheath fluid appears desirable in most droplet flow cytometers. To some extent this may be due to the fact that the required acceleration of the sheath fluid and pressure, and the resulting increase in the friction of the sheath fluid against nozzle container (2), each make a constant convergence desirable to avoid nonlaminar flow results. Basically it has been empirically found that through a continuously converging nozzle container optimal conditions for maintaining laminar flow can be created.

In addition to the aspect of maintaining laminar flow, the continuously converging nozzle container can provide amplification of the oscillations (11). Similar to horn and

other designs, the continuous convergence combines with the principals of conservation of energy so that the amplitude of the oscillations actually increases as it passes from piezoelectric crystal (10) to nozzle exit (6). This amplification may be maximized not only by positioning the oscillator at or near the largest cross-sectional area but also by making oscillator surface (18) to have an area substantially as large as the largest cross-sectional area. In this regard by "substantially" it is meant that the oscillator should be as large as practically possible after consideration of the typical desire to introduce substance through the center axis of nozzle volume (3) as well as this invention's unique desire to maintain oscillator side (21) spaced apart from nozzle container (2). The amplification may also be enhanced by providing for continuous convergence from sheath fluid port (4) through to nozzle exit (6). As mentioned earlier, each of the foregoing aspects also contribute to the present invention's extraordinary reduction in input power requirements.

To create oscillations (11), piezoelectric crystal (10) is powered through an alternating voltage source (22) as those skilled in the art can easily understand. Through the teachings of the present invention, alternating voltage source (22) may be configured to stimulate the oscillator with the voltage amplitude of less than 100 millivolts and thus represents orders of magnitude of reduction in the typical voltage applied to piezoelectric crystals in such systems. This voltage may be greater than 10 millivolts or so as that was a representative level at which droplet formation seems to occur. It should be understood, however, that this limitation should not be taken as a lower limit since the teachings of this invention may become refined and alternative designs may be developed which result in further reduction in power.

Yet another independent feature of the present invention is its design to allow the nozzle section to be easily replaced or cleaned while permitting laminar flow. Referring to figure 4,

it can be seen that the entire nozzle container (2) may be made of several components. Nozzle container (2) may consist of cap section (23) to which piezoelectric crystal (10) may be attached. Cap section (23) may be attached in some sealing fashion or may even be integral to nozzle body (24) as shown in figure 1. Similarly, nozzle body (24) may be sealingly attached to nozzle tip (25). Each of these seals may consist of O-rings as but one example of the types of seals shown in figure 4. Nozzle tip (25) may be a ceramic fabricated item which includes an exit situated at its tip. This exit may actually be an orifice made through techniques known by those skilled in the art (such as the use of tungsten wire and the like) so as to create a small orifice of about 50 to 150 microns in diameter.

Unlike the designs shown in the prior art such as those shown in figure 5, nozzle tip (25) need not be sealed to nozzle body (24) on its inner surface. Instead, the nozzle body inner surface (26) joins smoothly with the nozzle tip inner surface (27) at tip joint (28). This smooth transition is to the degree necessary to maintain laminar flow in the particular application. It can be achieved through the inclusion of edge insert (29) within nozzle body (24) so as to allow nozzle tip (25) to be inserted into nozzle body (24). In this fashion seal (30) can be positioned so as to contact the outer surface (31) of nozzle tip (25) and thus avoid any adverse impacts on laminar flow within nozzle volume (3). By locating seal (30) off of inner surface (27) of nozzle tip (25), the seal can be kept away from areas which are important to laminar flow. As may be understood, a great variety of designs may be accomplished to achieve this goal. Importantly, it should be understood that inner surface (27) of nozzle tip (25) is defined merely with respect to its function, namely, the surface which contacts and directs the flow of sheath fluid of nozzle volume (3). Further, the definition of "smooth" is also relatively defined as those transitions which do not significantly interrupt laminar flow and thus do not degrade the performance of the flow cytometer. It should also be understood that the seal between any two components such as

the seal between nozzle body (24) and nozzle tip (25) may be direct or indirect through the use of intervening materials or components.

Yet another independent aspect of the invention is the aspect of being able to adjust the location at which the substance is introduced. As mentioned earlier, those skilled in the art have long recognized the need to achieve variations in the entire process to accommodate variations in conditions practically experienced. As shown in figure 3, the present invention affords the ability to vary the rate at which substance is introduced without disrupting laminar flow and the like. This is achieved through positioning substance introduction port (9) within convergence zone (32) as may be easily understood and by varying the location of substance introduction port (9) within convergence zone (32). As shown, substance introduction port (9) may move along the primary flow direction to maintain an optimal relationship to the flow of the sheath fluid. Through this technique, the relative concentrations of the substance introduced and the sheath fluid can be varied. This can act to avoid the resolution drop and the like which the prior art appeared to consider unavoidable as they adapted to changing conditions.

Further, since it may be desirable to maintain equal velocities at substance introduction port (9), and since substance tube (33) may be moved, it is possible to include a controller (34) which receives signals from some type of sensor (14) and which may act to control a movement mechanism (35) and thus automatically adjust the location of substance introduction port (9) within nozzle container (2). Further, controller (34) may act to additionally control the pressure of substance reservoir (8) and sheath reservoir (5) for automatic correlation of the various factors based upon location or other parameters sensed. Since the theoretical relationship between these factors is well known for optimal conditions and since the programming or wiring of such a design could be easily achieved by those

skilled in the art, a variety of designs may be implemented to achieve this goal. Given the great variety of flow cytometer systems possible, it should be understood that a great variety of sensed values may be used ranging from concentration of the substance contained within substance reservoir (8), to the actual location of substance introduction port (9), to the pressure of the various sheath fluid or substance fluids, to some other property of the substance sensed by sensor (14). Each of these — or any combination of them and other factors — may be adjusted automatically to achieve desired relationships or to simply optimize results without regard to the actual predicted values. Naturally, in keeping with this broad concept it should be understood that sensor (14) may not be just one sensor but may in fact be a host of different sensors positioned at various locations depending upon the particular condition existing within the flow cytometer desired to be sensed. While, of course, the sensor (14) will only ascertain specific values, these values can indicate results which may be used to more appropriately adjust the location of the substance introduction port.

Similarly, a host of different designs for the location adjuster (shown in figure 3 as movement mechanism (35)) are possible. The location adjuster may also include some type of screw means (36), that is, some type of device which allows relatively continuous movement with fine adjustment. It may also include telescoping substance tube (37) (shown in figure 3 as potentially a redundant location adjuster for illustrative purposes only) or perhaps some type of slide design through the cap section. In applications in which the conditions remain relatively stable, a replacement substance tube of fixed length may also be provided. Thus, various substance tubes may be selected based upon the conditions encountered in that particular type of application. In this fashion, the limitation experienced by the prior art whereby variations in pressure were used but undesirably resulted in unequal fluid velocities at the location of substance introduction port (9) can be avoided. This affords an increase in the resolution.

The foregoing discussion and the claims which follow describe the preferred embodiment of the present invention. Particularly with respect to the claims and the broad concept discussed, it should be understood that changes may be made without departing from the essence of this patented invention. It is intended that changes are permissible to accommodate varying applications and will still fall within the scope of this patent. It is simply not practical to describe and claim all possible revisions nor is it practical to claim all combinations of the varying features. To the extent revisions utilize the essence of the present invention, each would naturally fall within the breath or protection encompassed by this path. This is particularly true for the present invention since its basic concepts and understandings are fundamental in nature and can be broadly applied. It is also particularly true since the present invention involves a number of potentially independent features which may be combined in synergistic ways for particular applications.

VI. CLAIMS

I claim:

1. A method of creating a droplet from a jet of a flow cytometer comprising the steps of:
   a. establishing a nozzle volume;
   b. introducing a flow of sheath fluid into said nozzle volume;
   c. introducing a flow of a substance within said sheath fluid in said nozzle volume;
   d. establishing an oscillator coupled to said nozzle volume;
   e. applying an alternating voltage with an amplitude of less than one hundred millivolts to said oscillator;
   f. allowing said sheath fluid to exit from said nozzle volume; and
   g. forming at least one droplet from said sheath fluid after allowing said sheath fluid to exit from said nozzle volume.

2. A method of creating a droplet from a jet of a flow cytometer as described in claim 1 wherein the amplitude of said alternating voltage is about ten millivolts.

3. A method of creating a droplet from a jet of a flow cytometer as described in claim 1 wherein said oscillator is established within said nozzle volume.

4. A method of creating a droplet from a jet of a flow cytometer as described in claim 3 wherein said nozzle volume has a perpendicular cross sectional area and wherein said oscillator is established substantially throughout said perpendicular cross sectional area.

5. A method of creating a droplet from a jet of a flow cytometer as described in claim 3 wherein said oscillator is unidirectionally coupled to said sheath fluid.

6. A method of creating a droplet from a jet of a flow cytometer as described in claim 1, 4, or 5 and further comprising the step of continuously converging said sheath fluid within said nozzle volume.

7. A system for creating a droplet from a jet of a flow cytometer comprising:
   a. a nozzle container establishing a nozzle volume and having a nozzle exit;
   b. a sheath fluid port located within said nozzle volume wherein said sheath fluid port introduces a sheath fluid;
   c. a substance introduction port located within said nozzle volume;
   d. an oscillator to which said sheath fluid is responsive;
   e. an alternating voltage source having an alternating voltage amplitude of less than one hundred millivolts connected to said oscillator; and
   f. a free fall area below said nozzle exit and within which said droplet forms.

8. A system for creating a droplet from a jet of a flow cytometer as described in claim 7 wherein said alternating voltage amplitude is about ten millivolts.

9. A system for creating a droplet from a jet of a flow cytometer as described in claim 7 wherein said oscillator is within said nozzle container.

10. A system for creating a droplet from a jet of a flow cytometer as described in claim 9 wherein said nozzle container has a cap section and wherein said oscillator comprises a piezoelectric crystal contained within said sheath fluid and attached to said cap section.

11. A system for creating a droplet from a jet of a flow cytometer as described in claim 10 wherein said sheath fluid port introduces a sheath fluid, wherein said oscillator has an oscillator surface which faces said sheath fluid, and further comprising an interface material between said oscillator surface and said sheath fluid which consists essentially of a protective coating.

12. A system for creating a droplet from a jet of a flow cytometer as described in claim 9 wherein said nozzle container has a largest perpendicular cross sectional area and wherein said oscillator is located at said largest perpendicular cross sectional area and is substantially as large as said largest perpendicular cross sectional area.

13. A system for creating a droplet from a jet of a flow cytometer as described in claim 10 wherein said oscillator has an oscillator side and further comprising a spacer which maintains said oscillator side detached from said nozzle container.

14. A system for creating a droplet from a jet of a flow cytometer as described in claim 7 or 12 wherein said nozzle container continuously converges.

15. A system for creating a droplet from a jet of a flow cytometer as described in claim 14 wherein said converging nozzle container continuously converges from said sheath fluid port to said nozzle exit.

16. A system for creating a droplet from a jet of a flow cytometer as described in claim 14 wherein said converging nozzle container comprises:

a. a nozzle body having an inner surface;

b. a nozzle tip having an inner surface; and

c. a seal located off of said inner surface of said nozzle tip and to which both said nozzle body and said

nozzle tip are responsive.

17. A method of creating a droplet from a jet of a flow cytometer comprising the steps of:
    a. establishing a nozzle volume;
    b. introducing a flow of sheath fluid into said nozzle volume;
    c. introducing a flow of a substance within said sheath fluid in said nozzle volume;
    d. initiating an oscillation within said nozzle volume;
    e. allowing said sheath fluid to exit from said nozzle volume; and
    f. forming at least one droplet from said sheath fluid after allowing said sheath fluid to exit from said nozzle volume.

18. A method of creating a droplet from a jet of a flow cytometer as described in claim 17 wherein said step of initiating an oscillation within said nozzle volume comprises the step of establishing an oscillator within said sheath fluid.

19. A method of creating a droplet from a jet of a flow cytometer as described in claim 17 wherein said oscillation passes through a material interface and further comprising the step of minimizing the number of material interfaces which said oscillation must pass through.

20. A method of creating a droplet from a jet of a flow cytometer as described in claim 19 wherein said oscillation is created by an oscillator and wherein said oscillation passes through only a protective coating on said oscillator before imparting on said sheath fluid.

21. A method of creating a droplet from a jet of a flow cytometer as described in claim 19 wherein the flow of said sheath fluid has a primary flow direction and wherein said

step of minimizing the number of material interfaces which said oscillation must pass through comprises the step of coupling said oscillation in substantially only said primary flow direction.

22. A method of creating a droplet from a jet of a flow cytometer as described in claim 17 and further comprising the step of directly transferring said oscillation to said sheath fluid.

23. A method of creating a droplet from a jet of a flow cytometer as described in claim 17, 18, or 22 and further comprising the step of unidirectionally applying an oscillation to said sheath fluid.

24. A method of creating a droplet from a jet of a flow cytometer as described in claim 23 and further comprising the step of directionally isolating said oscillation.

25. A method of creating a droplet from a jet of a flow cytometer as described in claim 17 and further comprising the step of directionally isolating said oscillation.

26. A method of creating a droplet from a jet of a flow cytometer as described in claim 24 wherein said step of isolating said oscillation comprises the step of coupling said oscillation to said sheath fluid along only one plane.

27. A system for creating a droplet from a jet of a flow cytometer comprising:

a. a nozzle container establishing a nozzle volume and having a nozzle exit;

b. a sheath fluid port located within said nozzle volume wherein said sheath fluid port introduces a sheath fluid;

c. a substance introduction port located within said nozzle volume;

d. an oscillator within said nozzle container; and
e. a free fall area below said nozzle exit and within which said droplet forms.

28. A system for creating a droplet from a jet of a flow cytometer as described in claim 27 wherein said sheath fluid port introduces a sheath fluid and wherein said oscillator comprises a piezoelectric crystal contained within said sheath fluid.

29. A system for creating a droplet from a jet of a flow cytometer as described in claim 27 wherein said sheath fluid port introduces a sheath fluid, wherein said oscillator has an oscillator surface which faces said sheath fluid, and further comprising an interface material between said oscillator surface and said sheath fluid which consists essentially of a protective coating.

30. A system for creating a droplet from a jet of a flow cytometer as described in claim 27 wherein said nozzle container comprises:
a. a cap section;
b. a nozzle body sealed to said cap section; and
c. a nozzle tip having said nozzle exit situated thereon, wherein said nozzle tip is sealed to said nozzle body, and wherein said sheath fluid flows through said nozzle tip.

31. A system for creating a droplet from a jet of a flow cytometer as described in claim 27 wherein said oscillator has an oscillator surface which faces said sheath fluid and wherein said oscillator surface is planar.

32. A system for creating a droplet from a jet of a flow cytometer as described in claim 27 or 28 wherein said sheath fluid port introduces a sheath fluid and further comprising a coupling which is only planar and which

couples said oscillator to said sheath fluid.

33. A system for creating a droplet from a jet of a flow cytometer as described in claim 32 and further comprising a directional isolator between said oscillator and said nozzle container.

34. A system for creating a droplet from a jet of a flow cytometer as described in claim 33 wherein said oscillator emits a predominant frequency and wherein said directional isolator is effective at said predominant frequency.

35. A system for creating a droplet from a jet of a flow cytometer as described in claim 33 wherein said oscillator has an oscillator side and wherein said directional isolator comprises a spacer which maintains said oscillator side detached from said nozzle container.

36. A method of creating a droplet from a jet of a flow cytometer comprising the steps of:

a. establishing a nozzle volume;
b. introducing a flow of sheath fluid into said nozzle volume;
c. introducing a flow of a substance within said sheath fluid in said nozzle volume;
d. unidirectionally applying an oscillation to said sheath fluid;
e. allowing said sheath fluid to exit from said nozzle volume; and
f. forming at least one droplet from said sheath fluid after allowing said sheath fluid to exit from said nozzle volume.

37. A method of creating a droplet from a jet of a flow cytometer as described in claim 36 wherein said step of unidirectionally applying an oscillation to said sheath fluid comprises the step of directionally isolating said

oscillation.

38. A method of creating a droplet from a jet of a flow cytometer as described in claim 37 wherein said step of isolating said oscillation comprises the step of coupling said oscillation to said sheath fluid along only one plane.

39. A system for creating a droplet from a jet of a flow cytometer comprising:

a. a nozzle container establishing a nozzle volume and having a nozzle exit;

b. a sheath fluid port located within said nozzle volume wherein said sheath fluid port introduces a sheath fluid;

c. a substance introduction port located within said nozzle volume;

d. a free fall area below said nozzle exit and within which said droplet forms;

e. an oscillator to which said sheath fluid is responsive; and

f. a unidirectional coupling which couples said oscillator to said sheath fluid.

40. A system for creating a droplet from a jet of a flow cytometer as described in claim 39 wherein said unidirectional coupling comprises a surface which is only planar.

41. A system for creating a droplet from a jet of a flow cytometer as described in claim 39 and further comprising a directional isolator between said oscillator and said nozzle container.

42. A system for creating a droplet from a jet of a flow cytometer as described in claim 41 wherein said oscillator emits a predominant frequency and wherein said directional

isolator is effective at said predominant frequency.

43. A system for creating a droplet from a jet of a flow cytometer as described in claim 41 wherein said oscillator has an oscillator side and wherein said directional isolator comprises a spacer which maintains said oscillator side detached from said nozzle container.

44. A method of creating a droplet from a jet of a flow cytometer comprising the steps of:

a. establishing a nozzle volume;
b. introducing a flow of sheath fluid into said nozzle volume;
c. continuously converging said sheath fluid;
d. introducing a flow of a substance within said sheath fluid in said nozzle volume;
e. allowing said sheath fluid to exit from said nozzle volume; and
f. forming at least one droplet from said sheath fluid after allowing said sheath fluid to exit from said nozzle volume.

45. A method of creating a droplet from a jet of a flow cytometer as described in claim 44 wherein said flow cytometer has a nozzle body and nozzle tip and wherein said step of continuously converging said sheath fluid comprises the step of establishing a smooth transition from said nozzle body to said nozzle tip.

46. A method of creating a droplet from a jet of a flow cytometer as described in claim 44 wherein said nozzle volume has a largest perpendicular cross sectional area and further comprising the step of applying an oscillation to said sheath fluid at said largest perpendicular cross sectional area.

47. A method of creating a droplet from a jet of a flow

cytometer as described in claim 46 wherein said step of applying an oscillation to said sheath fluid is accomplished substantially throughout said largest perpendicular cross sectional area.

48. A method of creating a droplet from a jet of a flow cytometer as described in claim 44 and further comprising the step of establishing an oscillator within said sheath fluid.

49. A method of creating a droplet from a jet of a flow cytometer as described in claim 48 wherein said oscillator establishes an oscillation and wherein said oscillation passes through a material interface and further comprising the step of minimizing the number of material interfaces which said oscillation must pass through.

50. A method of creating a droplet from a jet of a flow cytometer as described in claim 49 wherein said oscillation passes through only a protective coating on said oscillator before imparting on said sheath fluid.

51. A method of creating a droplet from a jet of a flow cytometer as described in claim 49 wherein the flow of said sheath fluid has a primary flow direction and wherein said step of minimizing the number of material interfaces which said oscillation must pass through comprises the step of coupling said oscillation in substantially only said primary flow direction.

52. A method of creating a droplet from a jet of a flow cytometer as described in claim 48 and further comprising the step of directly coupling said oscillator to said sheath fluid.

53. A method of creating a droplet from a jet of a flow

cytometer as described in claim 44, 48, 49 or 51 and further comprising the step of unidirectionally applying an oscillation to said sheath fluid.

54. A method of creating a droplet from a jet of a flow cytometer as described in claim 53 and further comprising the step of directionally isolating said oscillation.

55. A method of creating a droplet from a jet of a flow cytometer as described in claim 54 wherein said step of isolating said oscillation comprises the step of coupling said oscillation to said sheath fluid along only one plane.

56. A system for creating a droplet from a jet of a flow cytometer comprising:

a. a continuously converging nozzle container establishing a nozzle volume and having a nozzle exit;

b. a sheath fluid port located within said nozzle volume wherein said sheath fluid port introduces a sheath fluid;

c. a substance introduction port located within said nozzle volume; and

d. a free fall area below said nozzle exit and within which said droplet forms.

57. A system for creating a droplet from a jet of a flow cytometer as described in claim 56 wherein said continuously converging nozzle container comprises:

a. a continuously converging nozzle body having an inner surface;

b. a continuously converging nozzle tip having said nozzle exit situated thereon, wherein said continuously converging nozzle tip has an inner surface and is sealed to said nozzle body, and wherein said sheath fluid flows through said nozzle tip; and

c. a tip joint wherein said tip joint smoothly

transitions the inner surface of said nozzle body to the inner surface of said nozzle tip.

58. A system for creating a droplet from a jet of a flow cytometer as described in claim 56 wherein said converging nozzle container continuously converges from said sheath fluid port to said nozzle exit.

59. A system for creating a droplet from a jet of a flow cytometer as described in claim 56 wherein said nozzle container has a largest perpendicular cross sectional area and further comprising an oscillator to which said sheath fluid is responsive and which is located at said largest perpendicular cross sectional area.

60. A system for creating a droplet from a jet of a flow cytometer as described in claim 59 wherein said oscillator has an oscillator surface area and wherein said oscillator surface area is substantially as large as said largest perpendicular cross sectional area.

61. A system for creating a droplet from a jet of a flow cytometer as described in claim 56 and further comprising an oscillator within said converging nozzle container.

62. A system for creating a droplet from a jet of a flow cytometer as described in claim 61 wherein said oscillator comprises a piezoelectric crystal contained within said sheath fluid.

63. A system for creating a droplet from a jet of a flow cytometer as described in claim 62 wherein said oscillator has an oscillator surface which faces said sheath fluid, and further comprising an interface material between said oscillator surface and said sheath fluid which consists essentially of a protective coating.

64. A system for creating a droplet from a jet of a flow cytometer as described in claim 56 and further comprising:
    a. an oscillator to which said sheath fluid is responsive; and
    b. a coupling which is only planar and which couples said oscillator to said sheath fluid.

65. A system for creating a droplet from a jet of a flow cytometer as described in claim 61 or 63 and further comprising a coupling which is only planar and which couples said oscillator to said sheath fluid.

66. A system for creating a droplet from a jet of a flow cytometer as described in claim 64 and further comprising a directional isolator between said oscillator and said nozzle container.

67. A system for creating a droplet from a jet of a flow cytometer as described in claim 66 wherein said oscillator emits a predominant frequency and wherein said directional isolator is effective at said predominant frequency.

68. A system for creating a droplet from a jet of a flow cytometer as described in claim 66 wherein said oscillator has an oscillator side and wherein said directional isolator comprises a spacer which maintains said oscillator side detached from said nozzle container.

69. A method of creating a droplet from a jet of a flow cytometer comprising the steps of:
    a. establishing a nozzle volume;
    b. introducing a flow of sheath fluid into said nozzle volume;
    c. converging said sheath fluid in a convergence zone;
    d. introducing a flow of a substance at a location within said sheath fluid in said convergence zone;
    e. adjusting the location at which said substance is

introduced within said convergence zone;

f. allowing said sheath fluid to exit from said nozzle volume; and

g. forming at least one droplet from said sheath fluid after allowing said sheath fluid to exit from said nozzle volume.

70. A method of creating a droplet from a jet of a flow cytometer as described in claim 69 wherein said step of adjusting the location at which said substance is introduced within said convergence zone comprises the step of establishing the desired concentration of said substance relative to said sheath fluid.

71. A method of creating a droplet from a jet of a flow cytometer as described in claim 69 or 70 wherein said step of adjusting the location at which said substance is introduced within said convergence zone comprises the step of establishing laminar flow of said substance within said sheath fluid.

72. A method of creating a droplet from a jet of a flow cytometer as described in claim 69 and further comprising the step of conducting an analysis of said droplet and wherein said step of adjusting the location at which said substance is introduced within said convergence zone comprises the step of optimizing the results of said analysis.

73. A method of creating a droplet from a jet of a flow cytometer as described in claim 69 wherein said step of adjusting the location at which said substance is introduced within said convergence zone is automatic.

74. A method of creating a droplet from a jet of a flow cytometer as described in claim 72 wherein said step of adjusting the location at which said substance is

introduced within said convergence zone comprises the steps of:

a. sensing values representative of conditions within said flow cytometer; and

b. automatically moving said location based upon said sensed values.

75. A method of creating a droplet from a jet of a flow cytometer as described in claim 74 wherein said conditions are at least one of the following:

a. the pressure of said sheath fluid;

b. the pressure of said substance;

c. the location at which said droplet is formed;

d. the rate at which droplet is determined to contain some of said substance; or

e. a property of said substance.

76. A method of creating a droplet from a jet of a flow cytometer as described in claim 69 wherein said substance is introduced through a substance tube and wherein said step of adjusting the location at which said substance is introduced within said convergence zone comprises the step of replacing said substance tube.

77. A system for creating a droplet from a jet of a flow cytometer comprising:

a. a nozzle container establishing a nozzle volume and having a nozzle exit;

b. a sheath fluid port located within said nozzle volume wherein said sheath fluid port introduces a sheath fluid;

c. a flow convergence zone within said nozzle volume;

d. a substance introduction port located within said flow convergence zone;

e. a location adjuster to which said substance introduction port is responsive;

f. a free fall area below said nozzle exit and within

which said droplet forms.

78. A system for creating a droplet from a jet of a flow cytometer as described in claim 77 wherein said substance introduction port comprises a substance tube having a fixed length and wherein said location adjuster comprises a replacement substance tube.

79. A system for creating a droplet from a jet of a flow cytometer as described in claim 77 wherein said convergence zone has a primary flow direction and wherein said location adjuster comprises a screw means which moves said substance introduction port along said primary flow direction.

80. A system for creating a droplet from a jet of a flow cytometer as described in claim 79 wherein said location adjuster comprises a telescoping substance tube which moves said substance introduction port along said primary flow direction.

81. A system for creating a droplet from a jet of a flow cytometer as described in claim 77 and wherein said location adjuster comprises:

a. a sensor;

b. a controller responsive to said sensor; and

c. a movement mechanism responsive to said controller and wherein said substance introduction port is responsive to said movement mechanism.

82. A system for creating a droplet from a jet of a flow cytometer as described in claim 81 wherein said substance introduction port introduces a substance and wherein said sensor senses at least one of the following:

a. the pressure of said sheath fluid;

b. the pressure of a substance introduced at a location within said sheath fluid in said convergence zone;

c. the location at which said droplet is formed;
d. the rate at which droplet is determined to contain some of said substance; or
e. a property of said substance.

83. A system for creating a droplet from a jet of a flow cytometer comprising:
a. a nozzle body having an inner surface;
b. a nozzle tip having an inner surface;
c. a seal located off of said inner surface of said nozzle tip and to which both said nozzle body and said nozzle tip are responsive;
d. a sheath fluid port located within said nozzle volume wherein said sheath fluid port introduces a sheath fluid;
e. a substance introduction port located within said nozzle volume; and
f. a free fall area below said nozzle exit and within which said droplet forms.

84. A system for creating a droplet from a jet of a flow cytometer as described in claim 83 wherein said nozzle tip has an outer surface and wherein said seal contacts said outer surface of said nozzle tip.

85. A system for creating a droplet from a jet of a flow cytometer as described in claim 83 or 84 wherein said nozzle body has an inner surface and further comprising an edge insert on said inner surface of said nozzle body.

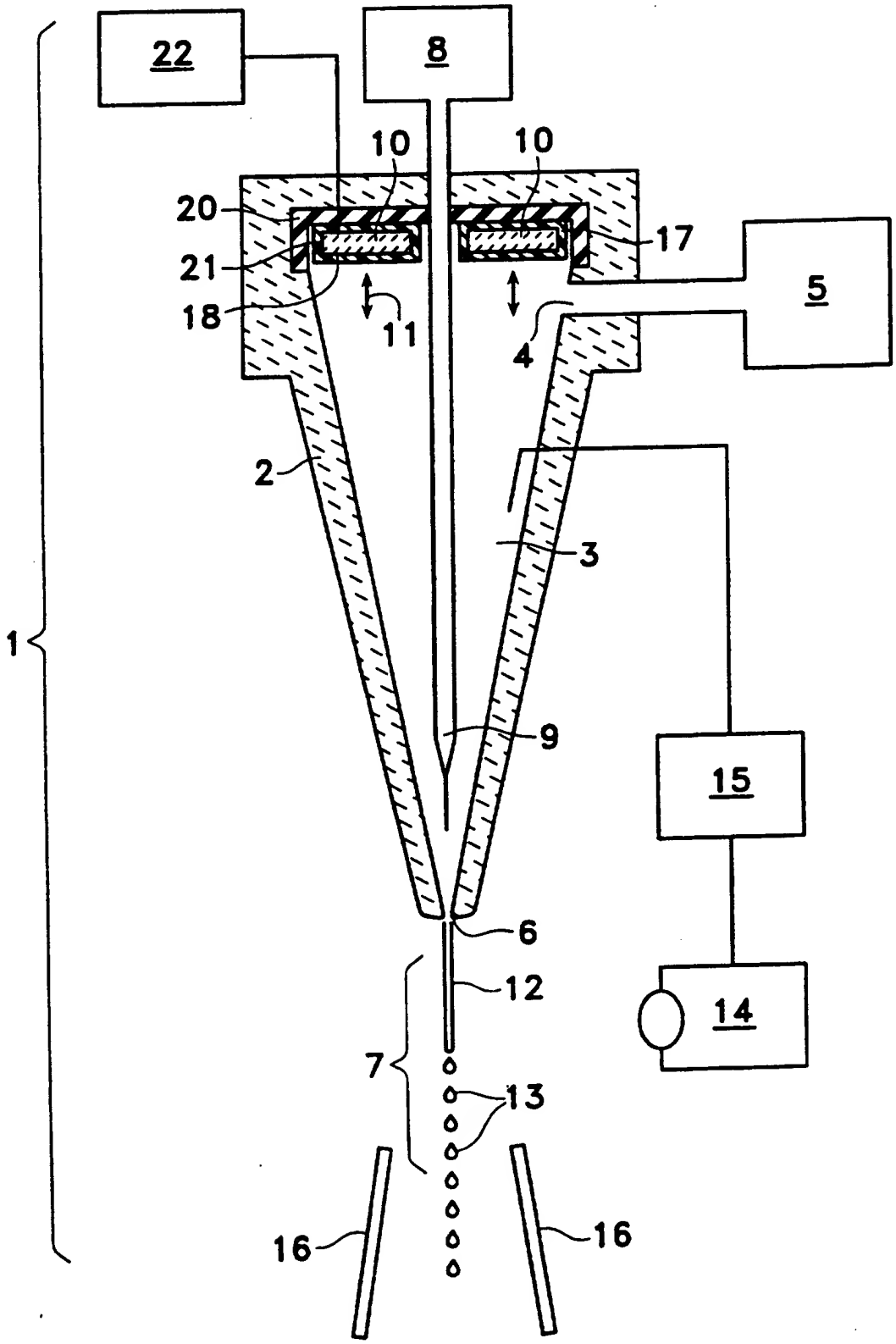

Fig. 1

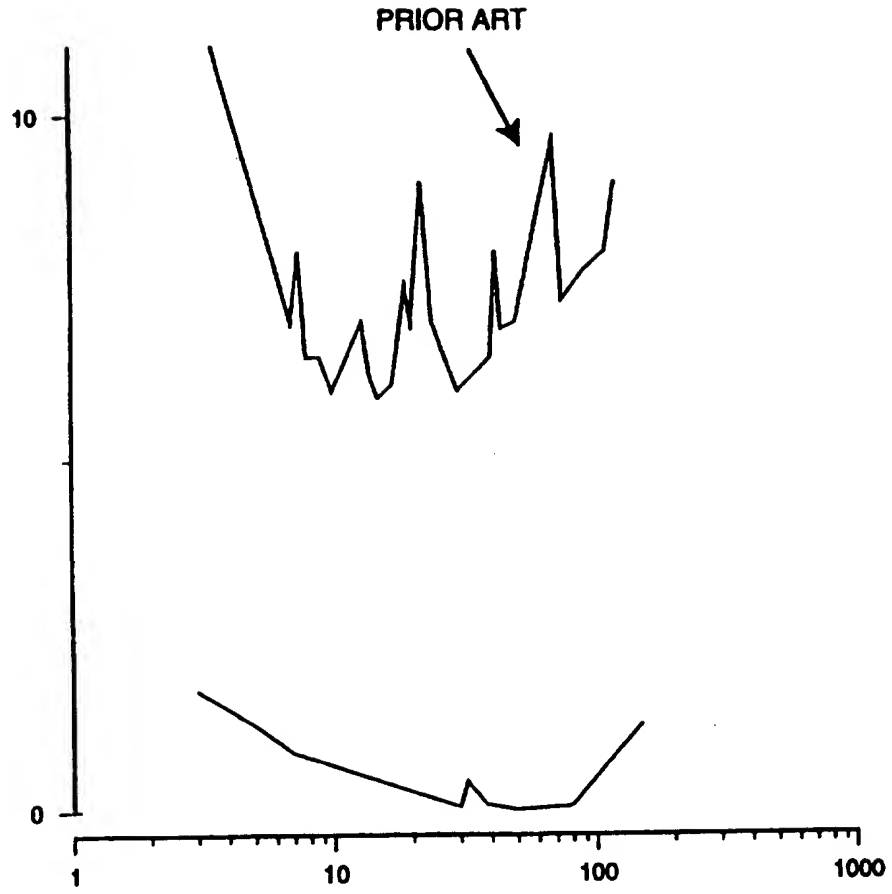

fig.2

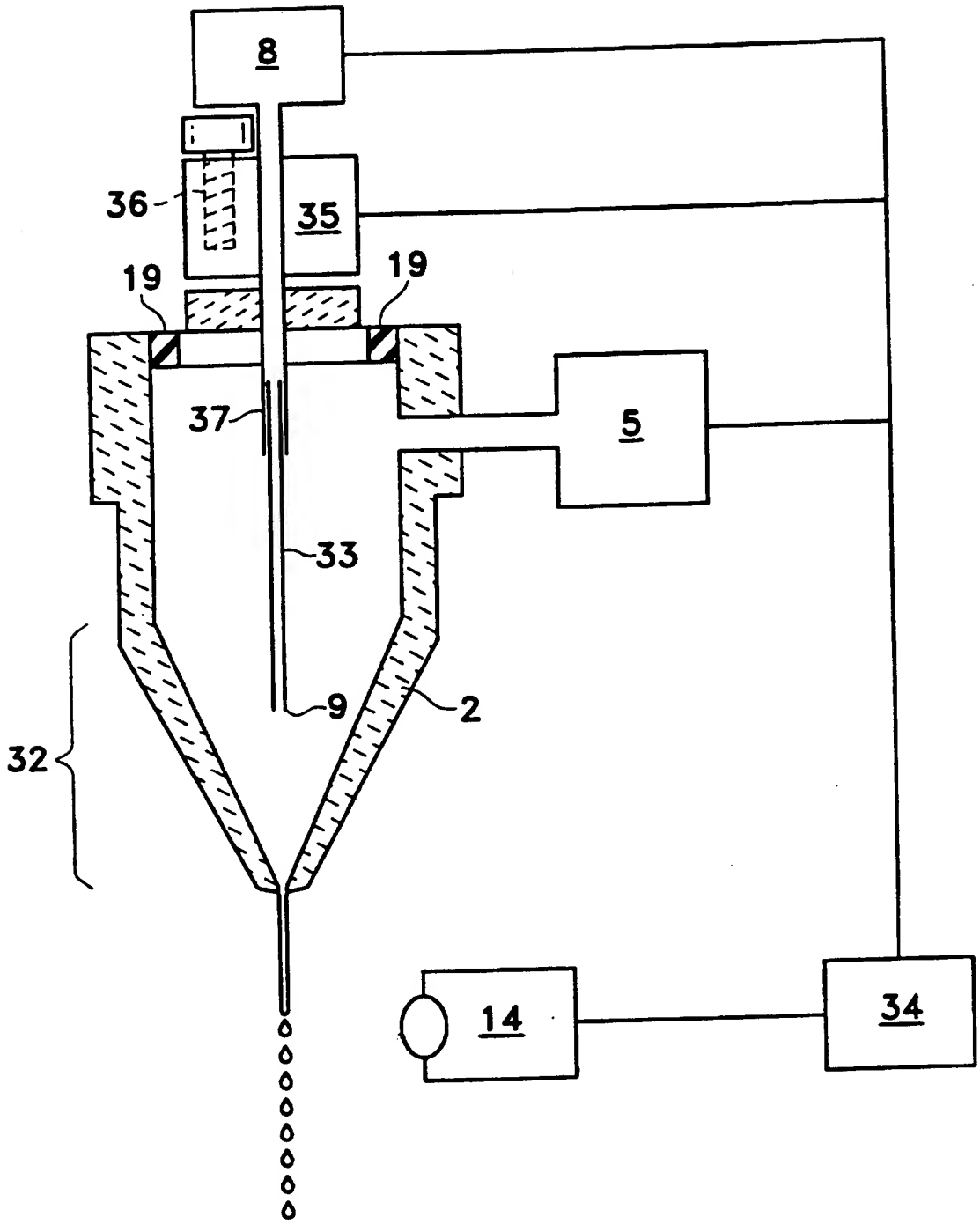

Fig. 3

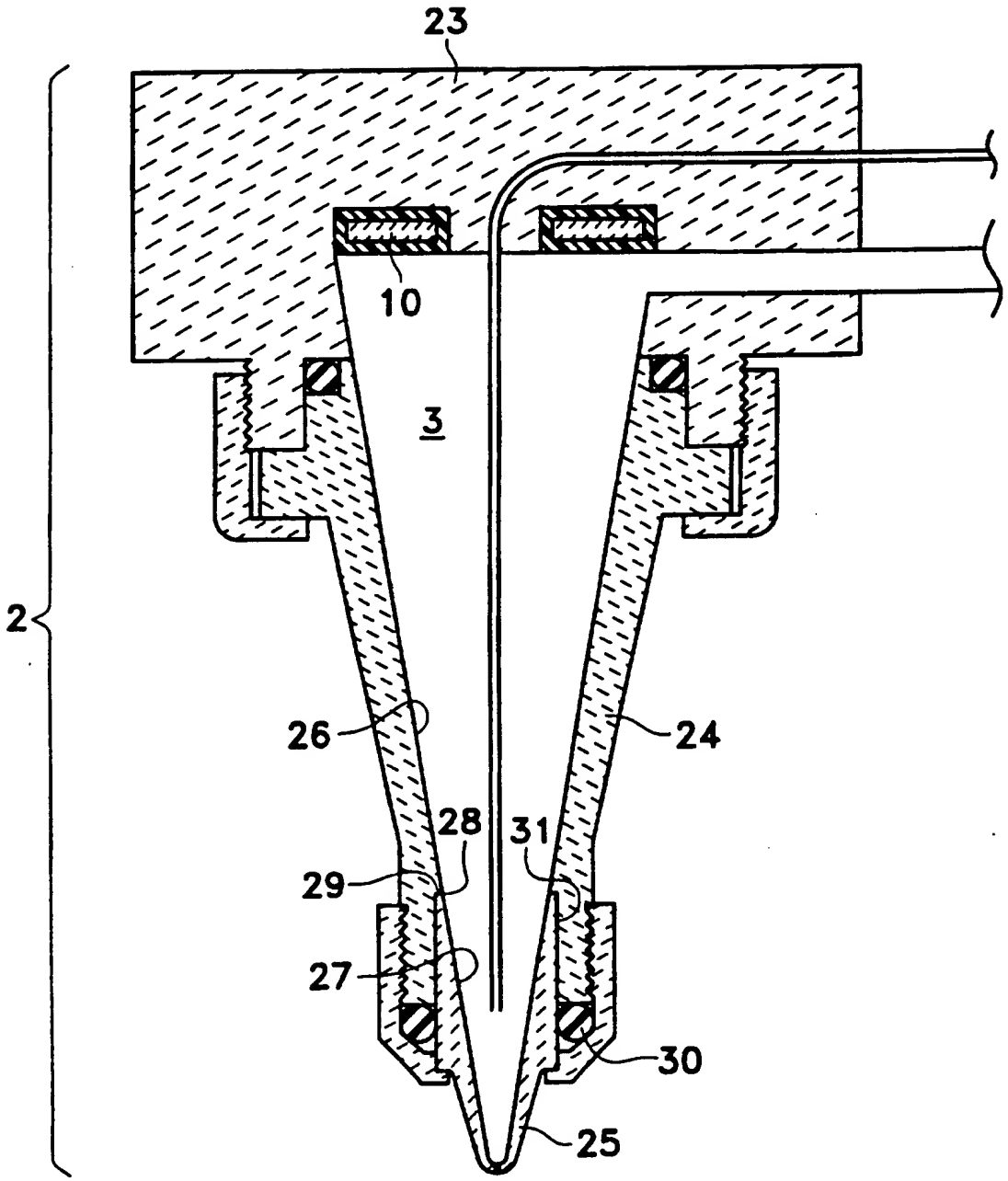

Fig. 4

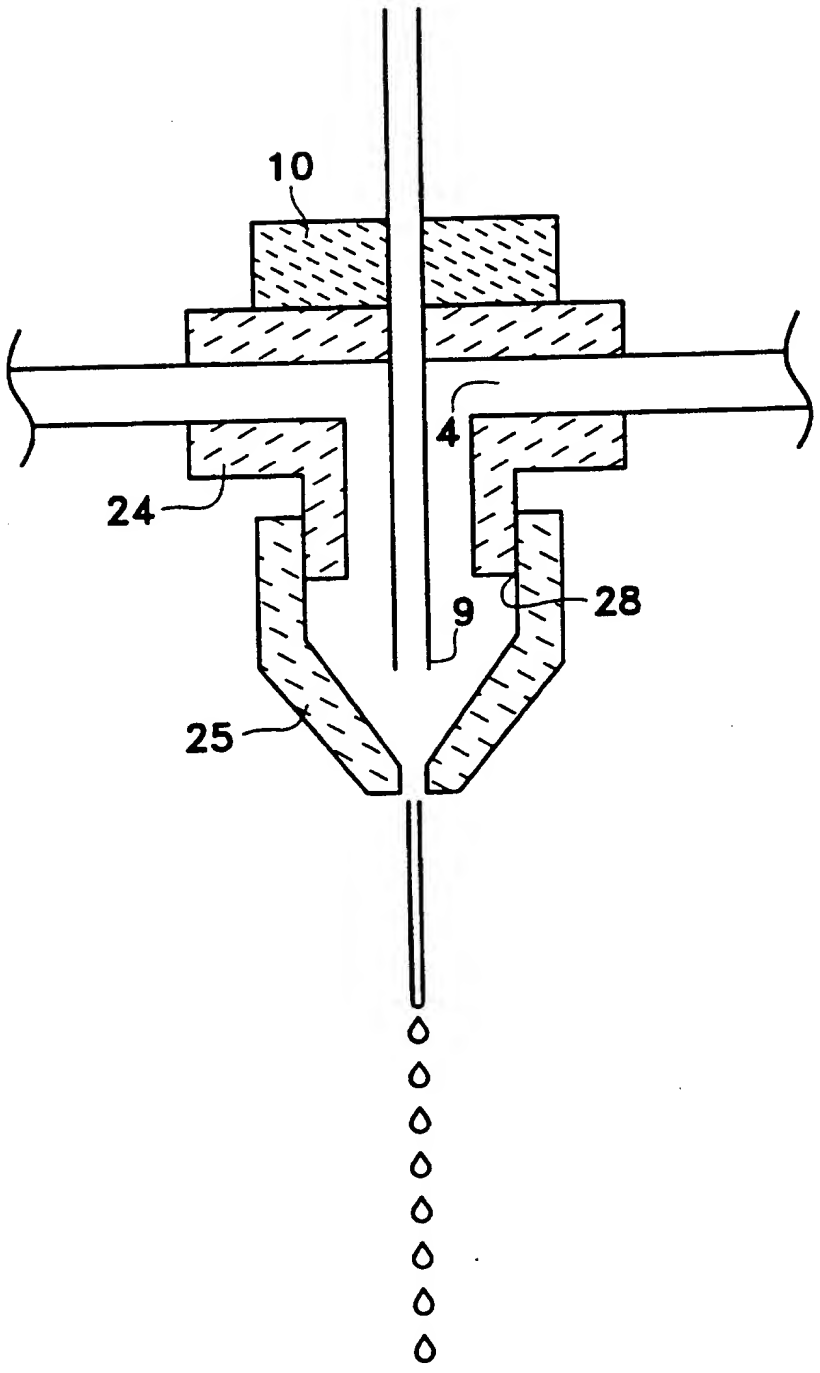

Fig. 5
(Prior Art)